PB-214 903

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October 1949

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PB 214 903

Federal Security Agency

Public Health Service

Environmental Health Center

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October 1949

A PROCEDURE FOR THE DETERMINATION OF CITRIC ACID IN WATER AND SEWAGE

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SUMMARY

The determination of 1 to 50 p.p.m. of citric acid in water and sewage is an adaptation of the micro method given by Natelson (1). The procedure consists of the bromination and oxidation of citric acid in aqueous solution to pentabromacetone which is then extracted with petroleum ether. An alkaline aqueous extraction of the ether solvent transfers the pentabromacetone to the water phase. The extractant buffered at pH 9.2 with borax and containing the reducing agent thiourea converts the pentabromacetone to a yellow color complex which is compared visually with permanent color standards or spectrophotometrically at a wave length of 440 millimicrons.

Reagents

1. Sulfuric acid, 1:1 dilution of concentrated H_2SO_4, C.P.
2. Saturated solution of C.P. bromine water.
3. Potassium bromide C.P. 11.9 gm. KBr/100 ml. (1 Normal).
4. Potassium permanganate C.P. 5 gm. $KMnO_4$/100 ml.
5. Hydrogen peroxide C.P. 3-6 per cent solution of H_2O_2.
6. Petroleum ether, boiling point 60-70°C. (purified).
7. Thiourea-borax reagent. Add 20 gm. $Na_2B_4O_7.10H_2O$, C.P. and 40 gm. $(NH_4)_2CS$ per liter. (The pH of this reagent should be 9.2).
8. Sodium hydroxide, C.P., 400 gm. NaOH/L.
9. Calcium chloride, saturated solution.

Purification of Petroleum Ether: The following method of purifying the ether may be used if necessary (1). Add 100 ml. concentrated H_2SO_4 per liter of petroleum ether. Shake and let stand for several days. Decant solvent and rewash with sulfuric acid. If acid becomes colored repeat the above washing until colorless. Decant solvent and add 10 ml. of a saturated solution of $KMnO_4$ in 0.5 N. H_2SO_4. Shake vigorously and add more $KMnO_4$ if necessary to maintain an excess (pink) oxidant. Allow separation of layers for 30 minutes and decant solvent. Wash solvent with distilled water until color and turbidity free. Dry solvent over anhydrous K_2CO_3 over night, filter and distill. (The lot of Skelly-solve B used at this laboratory was found sufficiently pure to allow omission of these purification steps).

Procedure

A. Purification and Bromination of Polluted Waters Containing Interfering Suspended or Volatile Matter.

To 100 ml. of sample or an aliquot diluted to 80 - 100 ml. containing 0.1 to 1.0, or a maximum of 4.0 mg. of citric acid, add 3 ml. of H_2SO_4. Evaporate by gentle boiling to 25-30 ml.; cool to room temperature; add 3 ml. of H_2SO_4 and 3 ml. of bromine water, and mix. Maintain excess bromine (yellow color) for 20 minutes by further additions of bromine water when necessary. If turbid, filter through a Gooch crucible. Dilute the filtrate to 50 ml. and transfer to a 125 ml. separatory funnel or glass-stoppered bottle. Proceed as in (D).

B. Purification of Sewage Containing Interfering Soaps, Fatty Acids, Suspended Matter and Oils.

Acidify 50 ml. sewage, or a portion diluted to this volume, to methyl orange with 1:1 sulfuric acid. Boil for one minute then cool under the tap. Add NaOH dropwise until alkaline to phenolphthalein, then add calcium chloride one drop at a time with mixing and settling until the last drop produces no further precipitate in the supernatant (about 2 drops). Stir gently several minutes and settle. Decant and filter through a prepared Gooch crucible under light suction. Wash flask with three 2 ml. portions of distilled water. Remove oil if the amount is significant by shaking the combined filtrate (which is alkaline to phenolphthalein) with two 25 ml. portions of carbon tetrachloride. Separate and discard the CCl_4 layers, then acidify with 6 ml. of H_2SO_4. Heat to remove remaining solvent and cool. Proceed as in (C) but omit the acid addition.

C. Bromination of Samples Requiring No Purification.

To 40 ml. of sample in a 125 ml. separatory funnel or glass-stoppered bottle add 6 ml. of H_2SO_4 and 3 ml. of bromine water, and mix. Maintain excess bromine for a minimum of 20 minutes. Proceed as in (D).

D. Oxidation of Brominated Citric Acid.

1. To the separatory funnel or bottle containing about 50 ml. of solution add 2 ml. of bromide reagent, mix and add $KMnO_4$ one drop at a time with mixing until a pink color persists for 15 seconds; then add 1 ml. of $KMnO_4$ to maintain a MnO_2 residual for 20 minutes.

2. Decolorize the MnO_2 slowly with H_2O_2 by dropwise additions and mixing until all of the brown precipitate disappears. (if the addition is too rapid bromine is liberated in the air phase and must be removed or destroyed with H_2O_2).

3. As a slight excess H_2O_2 may be added in D-2 it is immediately destroyed by adding $KMnO_4$ dropwise to a slight persistent yellow (bromine) followed by one drop of H_2O_2 to destroy the yellow color. (There should be less than one drop of H_2O_2 in excess before solvent extraction (E).)

E. Solvent Extraction.

1. Add 50 ml. of solvent to the separatory funnel and shake vigorously for 60 seconds. Allow layers to separate for several minutes before drawing off and discarding the water layer down to the clear ether.

2. Add gently 20 to 30 ml. of distilled water without shaking to rinse the funnel walls, discard the rinsings, repeat the rinsing of walls.

3. Wash the ether by shaking vigorously with 7-8 ml. of distilled water. Discard the wash water after separation on standing and repeat E-2.

4. Repeat E-3.

5. Repeat E-2.

6. Allow the pentabromacetone to age for at least two hours if a spectrometric analysis is to be made or preferably over night if a visual analysis is to be made.

F. Color Formation.

1. Transfer 10 ml. of ether solvent containing the pentabromacetone to a 50 ml. tall form Nessler tube and add 50 ml. of the borax-thiourea reagent.
2. Invert (do not shake) as rapidly as the air bubbles rise for three minutes to extract the pentabromacetone from the ether. The solvent should break up into fine globules which rise without being emulsified.
3. Allow the solvent to separate. Use an eye-dropper to remove and discard the solvent.

G. Colorimetric Comparison.

Twenty to thirty minutes after extracting the pentabromacetone in thiourea compare the color with permanent standards.

The permanent standards are prepared from stock solutions of ferric chloride and cobaltous chloride diluted to 50 ml. with 0.5 normal HCl in Nessler tubes. Solution A consists of 45.05 gm. of $FeCl_3.6H_2O$ dissolved in a total of one liter of 0.5 normal HCl. Solution B is prepared by diluting one part of solution A with nine parts of 0.5 normal HCl. Solution C contains 5.96 gm. of $CoCl_2.6H_2O$ per liter of 0.5 normal HCl. The color standards corresponding to citric acid are shown in the following table:

Stock Solution			Citric Acid Equivalent
A	B	C	
ml.	ml.	ml.	mg.
	0.7	0.6	0.005
	1.5	1.3	0.01
	2.9	2.4	0.02
	6.2	3.6	0.04
	11.0	4.3	0.06
	17.3	4.8	0.08
2.5		5.2	0.10
3.5		5.5	0.12
4.8		5.7	0.14
6.9		5.9	0.16
8.6		6.0	0.18
12.3		6.1	0.20

Calculations: The quantity of citric acid in mg. found in 10 ml. or 1/5 of the solvent extractant times 5000/ml. of original sample = p.p.m. citric acid.

H. Spectrophotometric Assay.

Fill a 10 cm. glass absorption cell with the thioureapentabromacetone color solution and determine its optical density 20-30 minutes after the thiourea-buffer extraction in a photometer at a wave length of 440 millimicrons. Thiourea-buffer shaken with petroleum ether is used as a blank to set the instrument at zero density. Compensation for turbidity of the samples may be made from the optical density of unknowns and blank at a wave length of 650 millimicrons.

An optical density at 650 mu. of over .01 is significant and the equivalent density at 440 mu. should be deducted. This correction on the Beckman Model DU spectrophotometer is:

Optical density at 650 mu. X 1.67 = equivalent density of the turbidity at 440 mu.

A calibration curve for citric acid based on 100 ml. of original sample and the use of 10 of the 50 ml. ether extract (1/5 of the total) for determining the density in a Beckman DU spectrophotometer at a wave length of 440 millimicrons using a 10 cm. glass absorption cell results in the following formula:

p.p.m. citric acid = corrected density X 12.25. If less than 100 ml. of original sample was taken the formula is: p.p.m. citric acid = corrected density times 1.225 times 1000 divided by the ml. of sample taken. If the intensity of color in the thiourea-buffer extract is too high a smaller aliquot of the petroleum ether should be extracted.

Interfering Substances

Acetone, glycogen, acid hematin, creatine, cholesterol, amytal, urea, taurine, toluene, acid digest casein, allantoin, and also uric, hippuric, lactic, malic, oxalic, tartaric, succinic, maleic, and fumaric acids are reported as giving no color with the method whereas 100 mg. quantities of beta hydroxybutyric acid and the ethyl ester of acetonedicarboxylic acid cause significant interference.

Reference

(1) Natelson, S., Pincus, J. B., and Lugovoy, J. K. J. Biol. Chem., 175, 745 (1948).